

# Isolation and characterization of human apolipoprotein A-IV from lipoprotein-depleted serum

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**Abstract** Human apolipoprotein A-IV is an acidic polypeptide of molecular weight 46,000 that is secreted into lymph on the surface of nascent chylomicrons, but which exists in circulation unassociated with lipoproteins. Previous studies of this protein have utilized material isolated from a  $d < 1.006$  g/ml fraction of human serum and from human lymph. Although it has been suggested that apoA-IV circulating in plasma is the product of dissociation from the surface of nascent chylomicrons, it has been characterized by immunological techniques only. We have isolated human apoA-IV on a preparative scale from lipoprotein-depleted serum using a technique of adsorption to a phospholipid-triglyceride emulsion, followed by delipidation and preparative gel electrophoresis. The molecular weight, pI, and amino acid composition of material thus prepared agree with literature values for apoA-IV derived from chylomicrons. We have determined that apoA-IV is a glycoprotein containing 6% carbohydrate by weight (mannose 1.8%, galactose 1.55%, N-acetyl glucosamine 1.55%, sialic acid 1.1%). Electroimmunoassay of human serum using a monospecific rabbit antibody to serum-derived apoA-IV found  $13.1 \pm 1.8$  mg/dl, a value in agreement with determinations using antibodies to chylomicron-derived apoA-IV. We conclude that apoA-IV may be easily purified from normal human serum, and that the material thus isolated has identical chemical, physical, and immunological properties to apoA-IV obtained from human lymph. It is therefore likely that the dissociation of apoA-IV from the surface of nascent chylomicrons following their entry into circulation is not attended by changes in the structure or composition of this apoprotein.—**Weinberg, R. B., and A. M. Scanu.** Isolation and characterization of human apolipoprotein A-IV from lipoprotein-depleted serum. *J. Lipid Res.* 1983. **24:** 52–59.

**Supplementary key words** Intralipid® • triglyceride-rich particles • electroimmunoassay • electroelution • electroanalysis

Apolipoprotein A-IV is an acidic polypeptide of molecular weight 46,000 that has been identified in several mammalian animal species (1–3) and in man (4–6). It is actively synthesized in the enterocytes of the small intestine during fat absorption and is thereafter incorporated onto the surface of nascent chylomicrons that are then secreted into lymph (2, 6, 7). Interestingly, in human plasma this apolipoprotein is found primarily in the lipoprotein-free fraction of density  $> 1.25$  g/ml, although small amounts are present in VLDL and HDL in nonhuman species (5, 6, 8). Studies of its metabolic behavior in rats (9) in chyluric patients (7), and in nor-

mal subjects (10) indicate that apoA-IV dissociates from the surface of nascent chylomicrons following their entry into plasma by an as yet undetermined mechanism. Hence, identification of apoA-IV in plasma has been by immunologic or radiotracer techniques only; moreover, it has not been established whether apoA-IV found in the fraction of density  $> 1.25$  is identical or structurally different from the protein found on the surface of chylomicrons. The metabolic function of this apolipoprotein is not established.

Most studies of apoA-IV have utilized mesenteric or thoracic duct lymph as a source of this protein. In humans, this necessitates access to patients with chyluria (7), chylous effusions (5), or hyperlipoproteinemias (4), diseases of relatively infrequent occurrence. We report here a novel technique for the isolation and purification of apoA-IV on a large scale from lipoprotein-depleted human serum, using adsorption to phospholipid-triglyceride emulsions followed by delipidation and preparative gel electrophoresis. The material thus obtained is physically, chemically, and immunologically identical to apoA-IV obtained from human lymph.

## METHODS

### Preparation of lipoprotein-depleted serum (LPDS)

All centrifugations were performed in a Beckman L8-70 ultracentrifuge at  $10^{\circ}\text{C}$  unless otherwise stated. Blood was obtained from normolipemic male donors following a 12-hr fast. The blood was allowed to clot at room temperature for 1 hr and the serum was then separated by low speed centrifugation. The serum was made 0.05% in Na EDTA (pH 7.0) and its density was raised to 1.25 g/ml by the addition of solid NaCl and NaBr. Centrifugation was performed in a Ti-60 rotor at 55,000 rpm for 48 hr. The floating lipoproteins were removed by aspiration and the remaining infranatants were pooled and dialyzed exhaustively against 0.15 M

Abbreviations: PBS, phosphate-buffered saline; LPDS, lipoprotein-depleted serum; TRP, phospholipid-triglyceride emulsion particles.

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NaCl, 0.05 M K-phosphate buffer, pH 7.4, 0.05% Na EDTA, pH 7.4 (henceforth referred to as PBS).

### Preparation of phospholipid-triglyceride emulsion particles (TRP)

Intralipid® was obtained from Cutter Laboratories and stored at 4°C. Aliquots (35 ml) were placed in polyallomer tubes and centrifuged in a Beckman SW-27 rotor for 35 min at 4°C. The semisolid floating cream layers were separated from the triglyceride-poor mesophases (11) by tube slicing, and resuspended in a sufficient amount of PBS to yield the original volume of Intralipid. Particles thus prepared were stored at 4°C and were used within 12 hr of preparation. Triglyceride concentration was  $8170 \pm 1875$  mg/dl; phospholipid concentration was  $653 \pm 28$  mg/dl ( $n = 3$ ).

### Incubation of LPDS and TRP

A series of small scale incubations were performed to determine the optimal conditions for the isolation of apoA-IV. For incubations at different pH values and TRP/LPDS ratios, aliquots of LPDS (4.5 ml) were diluted with different amounts of PBS and the pH was adjusted, as required, by the addition of solid KOH, or concentrated  $H_3PO_4$ . Varying amounts of TRP solution were then added to yield a final volume of 10 ml. For incubations at different ionic strengths, 4.5 ml of LPDS was diluted with 4.5 ml of PBS and solid NaCl was added to yield solutions of 0.15 to 4 M. The pH was readjusted to 7.4 by the addition of solid KOH prior to the addition of 1 ml of TRP solution. All mixtures were incubated at 37°C for 1 hr with gentle agitation. The incubations were terminated by immersion of the flasks in an ice-water bath.

Preparative scale incubations were performed in 4 M NaCl, at pH 7.4: 250 ml of LPDS was diluted with 250 ml of PBS, and 140 g of solid NaCl was added with stirring. The pH was readjusted to 7.4 with solid KOH, following which 100 ml of TRP solution was added. Incubation conditions were identical to those for the small scale studies.

### Reisolation and delipidation of TRP

Polyallomer tubes for the SW-27 rotor were filled with 30 ml of cooled incubation mixture and 5 ml of PBS was carefully layered on top of each tube. For the small scale incubations, polyallomer tubes for the SW-40 rotor were filled with 10 ml of incubation mixture and overlaid with 1 ml of PBS. Centrifugation was performed in a SW-27 rotor at 27,000 rpm or in a SW-40 rotor at 39,000 rpm for 35 min at 4°C. The TRP formed a semisolid cream layer at the top of each tube that could be cleanly separated from the LPDS infranant by tube-slicing. Recovery of triglyceride was 91.2%

$\pm 5.7\%$  ( $n = 12$ ). The TRP was delipidated with 50 volumes of diethylether–absolute ethanol 3:1 at 4°C, overnight. The protein precipitate was collected by low speed centrifugation and dried under vacuum. Crude protein yield for four preparative incubations was  $37.3 \pm 12$  mg.

### Preparative gel electrophoresis

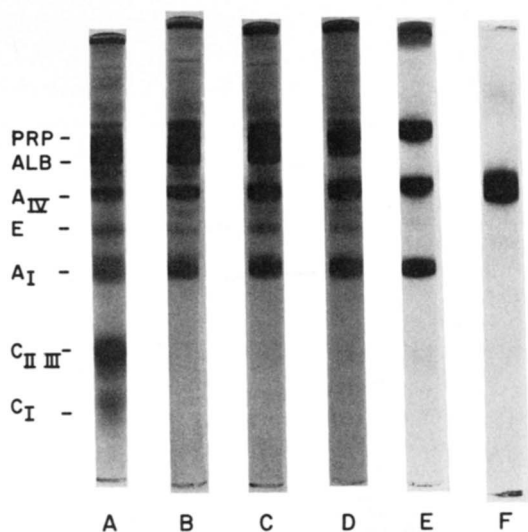
Preparative gel electrophoresis was performed using a Hoffer Model GT-14 apparatus, modified to recirculate buffer between the lower and upper chambers. Acrylamide (7.5%) in 0.12 M phosphate buffer, pH 7.20, with 0.1% SDS was cast in 15 mm  $\times$  130 mm rods. The dried precipitate was solubilized in 0.05 M Tris, pH 8.6, with 1% SDS and 1% mercaptoethanol, and was incubated for 1 hr at 37°C. One milligram of protein was placed on top of each gel and electrophoresis was then performed with 0.05 M phosphate running buffer, pH 7.2, at 25 mA/tube for 15 hr. The gels were freed from the glass tubes and placed under UV light which allowed visualization of the protein bands by weak fluorescence. The protein bands were cut from the gels and were inserted into glass tubes with a constriction at the bottom, allowing attachment of a dialysis sac filled with 18 mM Tris-borate buffer, pH 8.3, 0.5 mM EDTA, 0.1% SDS. The tubes were inserted back into the Hoffer apparatus and electrolyte was placed in both chambers.

Electroelution was carried out at 150 V for 12 hr at room temperature. At the end of this period the protein could be found concentrated in the bottom of the dialysis sacs. No detectable protein remained in the acrylamide disks as assessed by staining with Coomassie blue. Electroelution was then carried out by replacing the electrolyte-SDS solution with electrolyte free of SDS, and increasing the voltage to 500 volts. After 2 days, 98.3%–99.8% of the SDS was removed as assessed by the use of tracer amounts of  $^{35}S$ -labeled SDS. The SDS-free protein solutions were then dialyzed against 0.005 M  $NH_4HCO_3$ , pH 8.6, for 24 hr at 4°C, and lyophilized. The protein powders were stored under  $N_2$  at  $-70^\circ C$ .

### Production of antisera

Male New Zealand white rabbits weighing 6–8 kg were immunized with 500  $\mu g$  of apoA-IV prepared by slicing disks from analytical SDS acrylamide gels. The disks were emulsified with an equal volume of Freund's complete adjuvant and administered subcutaneously at multiple sites. Booster immunizations with 250  $\mu g$  of antigen were given at 2-week intervals until serum yielded a strong immunoprecipitate line against purified apoA-IV on Ochterlony plates. The rabbits were bled by the ear vein and the antiserum was fractionated by ammonium sulfate precipitation. The resulting gamma





**Fig. 1.** Analytical SDS polyacrylamide electrophoresis in 7.5% gels of delipidated TRP following incubation with A, whole human serum; B, LPDS dialyzed against PBS; C, LPDS, 1 M NaCl; D, LPDS, 2 M NaCl; E, LPDS, 4 M NaCl; F, purified apolipoprotein A-IV. Fifty  $\mu$ g of protein solubilized in 5 mM Tris, 1% SDS, 1% mercaptoethanol was applied to each gel. Apoprotein bands are identified along the right.

globulin fraction was dissolved in 0.05 M barbital buffer with 0.04% sodium azide and stored in small aliquots at  $-70^{\circ}\text{C}$ .

The antiserum did not react with purified apoA-I, apoA-II, apoE, apoB, Lp(a), or apoVLDL. Weak precipitin lines were noted with delipidated human chylomicrons and HDL.

#### Analytical procedures

Protein concentration in solutions was determined by the method of Lowry et al. (12) using bovine serum albumin as a standard. Phospholipid was determined by the method of Bartlett (13). Triglyceride determinations were performed with a Technicon autoanalyzer (14). Analytical polyacrylamide SDS gel electrophoresis in 7.5% gel and analytical alkaline urea gel electrophoresis in 7.5% gel were performed as previously described (15). Scanning of gels was performed on a Varian DMS-90 at 540 nm. Each gel was loaded with 50  $\mu$ g of protein. No correction was made for differences in protein chromogenicity. Analytical isoelectric focusing was performed in a 7.5% polyacrylamide gel with 8 M urea and 2.0% pH 4–6 ampholine according to the method of Gidez, Swaney, and Murnane (16). The pH gradient was determined in unstained gels using a BioRad pHroFiler<sup>®</sup>. Amino acid analysis was performed on a Dionex D-502 amino acid analyzer following the general procedure of Spackman, Stern, and Moore (17). For analysis of apoA-IV, hydrolysis of protein samples was carried out at  $112^{\circ}\text{C}$  in vacuo for 24, 48, and 72

hr. The threonine and serine content at time zero was determined by extrapolation. Cysteine content was determined by analysis of samples treated with hydrogen peroxide following hydrolysis. Tryptophan content was determined by the method of Edelhoch (18). Analysis for carbohydrate was performed by the method of Dawson (19) on a Varian Aerograph 2100 gas-liquid chromatograph using a 3% OV-1 column. Samples were hydrolyzed with 1 N HCl in methanol, neutralized with silver carbonate, and trimethylsilylated before injection. Mannitol was used as an internal standard. Electroimmunoassay for apoA-IV was performed using the method of Utermann and Beisiegel (5): 1% agarose gels in 0.05 M barbital buffer incorporating 4% antibody and 0.05% Triton X-100 were poured on plastic plates. The final gel dimensions were  $1.5 \times 93 \times 84$  mm. Standards and samples were prepared in 0.05 M barbital buffer, pH 8.6, with 2% Triton X-100. Serum samples were diluted 1/4, infranatants were diluted 1/2. The gels were run for 4 hr at 10 V/cm, pressed, rinsed, dried, stained, and destained as described. Rocket heights were measured on graph paper. A linear standard curve between 0.5 and 8 mg/dl was obtained. Immunodiffusion was performed in precast agarose gel plates in 0.05 M barbital buffer, pH 8.6. Measurements of apoA-IV fluorescence were made with a Perkin-Elmer MPF-44B fluorescence spectrophotometer using a 1-cm quartz cell.

#### RESULTS

The incubation of TRP with whole serum resulted in the transfer of several apoproteins onto these fat particles with the predominant species being the C-proteins. The particles also contained large amounts of albumin. The amount of apoA-IV present was 6.1% of the total recovered protein as assessed by densitometry of polyacrylamide gels (**Fig. 1A**). The incubation of TRP with LPDS resulted in the uptake of apoA-I, apoE, apoA-IV, albumin, and higher molecular weight proteins, but not the C-proteins (**Fig. 1B**). In these experiments, apoA-IV constituted 16.0% of the protein taken up by TRP. The uptake of apoA-IV was pH-dependent. At  $\text{pH} < 7.0$  the affinity of apoA-IV was reduced in relation to other proteins. Consequently, all further studies were carried out at  $\text{pH} 7.4$ . The amount of TRP solution added to the LPDS buffer mixture influenced the extraction of apoA-IV from LPDS, as assessed by electroimmunoassay of the post-incubation ultracentrifugal subnatants. At a TRP/LPDS ratio of 1:9, 57% of the apoA-IV in the incubation mixture was removed; at a 1:4.5 ratio, 82.9% was removed. A maximum extraction of 91.4% occurred with ratios greater than 1:3. Large scale preparative incubations were performed at a ratio

of 1:2.5. The addition of mercaptoethanol to the incubation media at a concentration of 1% did not alter apoA-IV binding to TRP, nor did the presence of the LCAT inhibitor N-ethyl maleimide at a concentration of 10 mM.

Increasing the ionic strength of the incubation buffer had a significant effect on protein binding to the TRP surface. As the molarity of NaCl increased, binding of higher molecular weight proteins—especially albumin—decreased; the binding of apoA-I, apoA-IV, and PRP was not affected (Fig. 1C, D). In 1 M NaCl, the percentage of apoA-IV of the protein recovered from delipidated TRP increased from 16.0% to 19.6%; it was 24.9% in 2 M NaCl and 28.1% in 3 M NaCl. In 4 M NaCl, the protein recovered from TRP consisted of a mixture of three proteins (Fig. 1E) with apoA-IV comprising 32.4% of the total.

These preliminary studies established that a maximum extraction of apoA-IV from LPDS occurs at pH 7.4 in 4 M NaCl at a LPDS/TRP ratio of greater than 1:3. Moreover, under these conditions, delipidation of recovered TRP yielded a mixture of only three apoproteins. Attempts to separate these proteins on gel filtration columns were not successful; thus, preparative gel electrophoresis was utilized. The localization of the protein bands in the gels was greatly facilitated by the

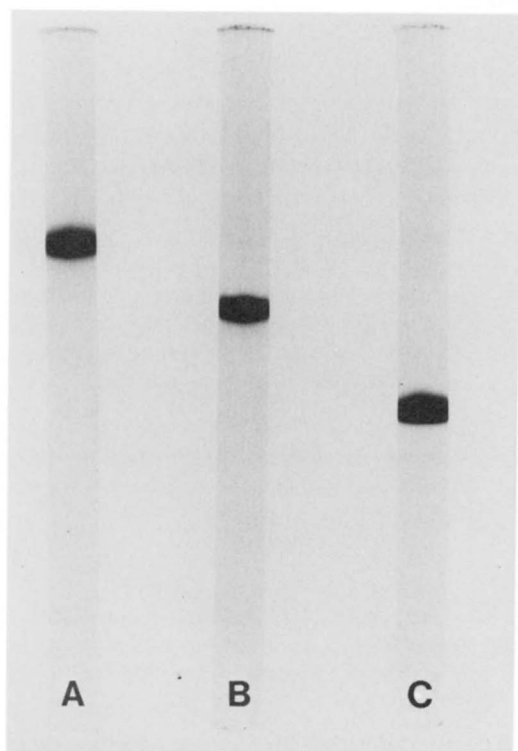


Fig. 2. Analytical SDS polyacrylamide electrophoresis on 7.5% gels of electroeluted apoproteins. A, Proline-rich protein; B, apolipoprotein A-IV; C, apolipoprotein A-I. Twenty  $\mu$ g of protein was applied to each gel in the presence of 1% mercaptoethanol.

TABLE 1. Amino acid composition of human apolipoprotein A-IV isolated from LPDS<sup>a</sup>

	mol/mol
Asparagine	33.0 $\pm$ 0.8
Threonine	14.4 $\pm$ 0.1
Serine	18.2 $\pm$ 0.2
Glutamine	82.8 $\pm$ 2.3
Proline	10.7 $\pm$ 0.4
Glycine	18.8 $\pm$ 0.5
Alanine	29.8 $\pm$ 0.8
Half-cysteine	1.8 $\pm$ 0.2
Valine	18.8 $\pm$ 0.5
Methionine	2.8 $\pm$ 0.3
Isoleucine	5.6 $\pm$ 0.2
Leucine	47.2 $\pm$ 1.4
Tyrosine	6.9 $\pm$ 0.3
Phenylalanine	9.4 $\pm$ 0.3
Lysine	25.0 $\pm$ 0.7
Histidine	7.3 $\pm$ 0.3
Arginine	23.3 $\pm$ 0.7
Tryptophan	6.0 $\pm$ 0.2

<sup>a</sup> Mean value of six determinations  $\pm$  S.D. Results reported as moles of amino acid per mole of protein.

finding that protein bands in untreated gels exhibited weak fluorescence under shortwave UV light. Preparative gels marked under UV light and subsequently stained with Coomassie blue showed that this technique could accurately and precisely locate the narrow (5 mm) apoprotein bands.

Other workers have recovered apoA-IV from preparative gel disks by elution in large volumes of buffer (9). However, we found that the technique of electroelution rapidly and completely removed all protein from the disks. In a subsequent step, electro dialysis allowed 98.3–99.2% removal of SDS in 48 hr from each protein specimen as assessed by counting <sup>35</sup>S-labeled SDS added to the dialysis sacs prior to the beginning of dialysis. The overall yield of apoA-IV was 20% of that present in the initial incubation mixture.

Each of the proteins so eluted gave a single band on analytical SDS gels (Fig. 2). Comparison of  $R_f$  values with those of standards of known molecular weight allowed calculation of molecular weights of 75,300, 45,800, and 25,800 for the three protein bands. Amino acid analysis, double immunodiffusion, and alkaline urea PAGE of the lower molecular weight protein showed it to be apolipoprotein A-I. Amino acid analysis and alkaline urea PAGE of the highest molecular weight protein were consistent with the proline-rich apoprotein first isolated by Sata et al. (20).

The amino acid analysis of the 45,800 molecular weight protein was consistent with apolipoprotein A-IV (Table 1 and Table 2). Treatment with mercaptoethanol did not alter the apparent molecular weight. By isoelectric focusing on 7.5% polyacrylamide in 8 M urea, its isoelectric point was 5.12 (Fig. 3); several minor



TABLE 2. Amino acid composition of human apolipoprotein A-IV<sup>a</sup>

Amino Acid	ApoA-IV Isolated from			
	LPDS	Lymph <sup>b</sup>	Hyperlipidemic Plasma <sup>c</sup>	Chylous Urine <sup>d</sup>
Asparagine	9.1	10.0	9.7	9.3
Threonine	4.0	3.6	4.5	5.8
Serine	5.0	5.1	7.3	9.2
Glutamine	22.9	23.0	22.5	17.4
Proline	3.0	3.7	3.4	3.8
Glycine	5.2	4.7	6.2	4.5
Alanine	8.2	8.2	8.2	9.4
Half-cysteine	0.5	nd <sup>e</sup>	nd	nd
Valine	5.2	4.6	5.1	6.2
Methionine	0.8	0.7	1.2	1.9
Isoleucine	1.6	1.5	2.0	1.6
Leucine	13.1	13.6	11.5	10.0
Tyrosine	1.9	2.2	1.6	2.9
Phenylalanine	2.6	3.0	3.1	4.4
Lysine	6.9	6.8	5.9	8.0
Histidine	2.0	2.0	2.2	1.4
Arginine	6.4	6.2	5.3	4.0
Tryptophan	1.7	nd	nd	nd

<sup>a</sup> Expressed as mol %.

<sup>b</sup> Weisgraber et al. (3).

<sup>c</sup> Beisiegel and Utermann (4).

<sup>d</sup> Green et al. (7).

<sup>e</sup> Not determined.

isoforms of pI 5.18, 5.16, 5.05, and 4.98 were also present as observed by Utermann and Beisiegel (5). The protein contained 6% carbohydrate by weight (mannose, 1.8%; galactose, 1.55%; N-acetyl glucosamine, 1.55%; sialic acid, 1.1%). Expressed as moles of carbohydrate per mole of protein we found: mannose 4.6, galactose 3.9, N-acetyl glucosamine 3.2, and sialic acid 1.8. The apoA-IV thus prepared did not cross-react with monospecific antiserum to apoA-I, apoA-II, apoB, apoE, Lp(a), or LDL.

Electroimmunoassay of normal human plasma using antibodies raised against apoA-IV prepared as described, yielded a mean value of  $13.1 \pm 1.8$  mg/dl ( $n = 20$ ). The intra-assay coefficient of variation was 2.7%; the interassay coefficient of variation was 7.6%. This value is in accord with determinations by Green et al. (6) and by Utermann and Beisiegel (5) who used antibodies raised against chylomicron-derived apoA-IV. The lowest value obtained (9.4 mg/dl) was in an individual with hypobetalipoproteinemia (total cholesterol 73 mg/dl). Studies of the fluorescence properties of apoA-IV showed a maximum emission wavelength of 420 nm. The peak excitation wavelength was 335 nm.

## DISCUSSION

The requirement of large quantities of mesenteric lymph as a starting material for the preparation of hu-

man apolipoprotein A-IV has undoubtedly been a factor contributing to the paucity of knowledge regarding the physical properties and metabolic functions of this protein. We report here a simple method for the extraction and purification of apoA-IV from human serum, which can provide a more readily available source of this protein for further study.

Triglyceride-rich particles prepared from Intralipid have been shown to have affinity for all classes of human apolipoproteins (20–23). In the course of studies on the interaction of TRP with human HDL and plasma transfer proteins (24), we observed that TRP isolated from control incubations containing only LPDS had acquired primarily apoA-I, but also small amounts of apoA-IV. This suggested to us that under appropriate conditions, TRP could be used as a vehicle to extract apoA-IV from human serum on a preparative scale. A similar approach was used by Sata et al. (20) in the purification of human proline-rich protein. Hence we sought conditions that would maximize the yield of apoA-IV.

When TRP is incubated with whole serum, the predominant apoproteins acquired are of the C-family; the percentage of apoA-IV is quite low. ApoC proteins bind most avidly to TRP, and if sufficient amounts are available they will saturate the particle surface, precluding the binding of apoproteins of the A class (25). ApoC

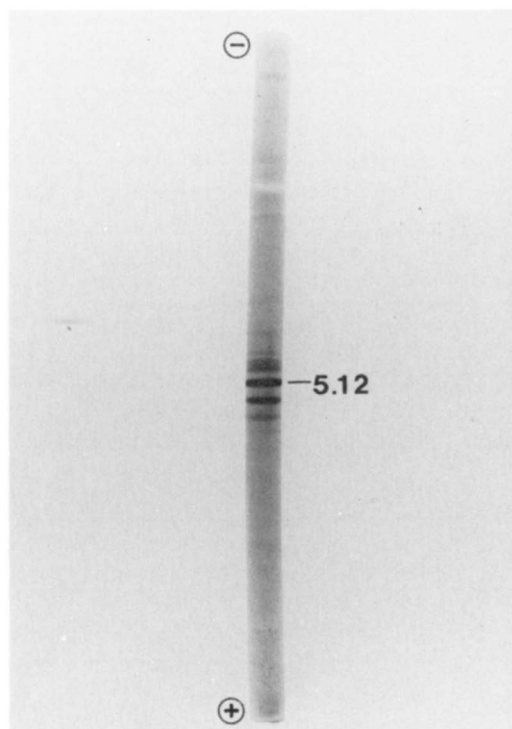


Fig. 3. Analytical isoelectric focusing of apoA-IV in 7.5% gel in the presence of 8 M urea. The gel was 2.0% pH 4–6 ampholine. The pH gradient was determined on an unstained duplicate gel (curve not shown). Twenty-five  $\mu$ g of protein was applied to the gel.

will even displace A apoproteins already present on the surface of TRP (26). Hence, a first essential step in the preparation of apoA-IV was the removal of the C-protein pool from serum by flotation of all lipoproteins. The LPDS thus prepared contained greater than 95% of the original apoA-IV, as this protein is not normally associated with lipoproteins (4–6).

Use of LPDS in incubations with TRP increased the proportional binding of apoA-IV, but several proteins of higher molecular weight, particularly albumin, were also bound to a great degree. Techniques for “washing” the recovered TRP, such as repeated ultracentrifugal flotations or passage through agarose columns, are effective in removing albumin and loosely bound higher molecular weight proteins, but unfortunately also result in the loss of apoA-IV (4, 5). We therefore utilized the stratagem of increasing the ionic strength of the incubation to reduce “nonspecific” protein binding to the TRP. Under such conditions, amphiphilic apolipoproteins bind to the hydrophobic TRP surface, whereas other hydrophilic plasma proteins remain in solution. This procedure allowed the adsorption of only three apoproteins to the TRP surface and increased the percentage yield of apoA-IV as well. The use of a single ultracentrifugation for recovery of the TRP minimized protein loss by dissociation.

ApoA-IV prepared by the above method demonstrated physical and immunological properties identical to those reported for apoA-IV isolated from chylomicrons (Table 3). We report, for the first time, a complete amino acid analysis of this apoprotein (Table 1), which is in agreement with other published determinations (Table 2). Of interest is that apoA-IV contains six res-

idues of tryptophan and two residues of cysteine per molecule. A novel finding is that apoA-IV is a glycoprotein that contains carbohydrate residues consistent with a single oligosaccharide side chain per molecule. This is perhaps not unexpected, for most circulating plasma proteins are glycoproteins and, as noted, apoA-IV in plasma is unassociated with lipoproteins. The presence of the oligosaccharide side chain may provide an explanation for the microheterogeneity demonstrated by isoelectric focusing.

The two other apoproteins recovered from LPDS by this method have previously been shown to have an affinity for TRP (18–21). The presence of free apoA-I in LPDS is likely due to dissociation from HDL by the high salt concentrations and centrifugal fields used to prepare LPDS (27). Proline-rich protein is normally found free in plasma unassociated with lipoproteins and has been purified by Sata et al. (20). Their procedure also utilized TRP, however, they did not recover apoA-IV from LPDS along with PRP. The reasons for this are not clear, but their use of unbuffered incubation conditions and agarose column chromatography for TRP recovery may have resulted in a low affinity of apoA-IV for TRP, with subsequent loss of this protein from the TRP surface.

We found it convenient to use preparative gel electrophoresis to purify apoA-IV from the mixture of three proteins obtained following delipidation of TRP. However, a reasonable separation of these three proteins is possible using alkaline urea PAGE (Fig. 4), and may therefore be feasible using anion exchange gel-chromatography. The combined electroelution-electrodialysis step was most useful in obtaining a concentrated

TABLE 3. Properties of apolipoprotein A-IV

Properties	Found	Literature
Molecular weight	45,800	46,000 (3, 4, 7)
Cleavage by mercaptoethanol	none	none (3–5)
pI	5.12 <sup>c</sup>	5.15 (5)
E <sub>280</sub> <sup>a</sup>	41,300	N.D. <sup>d</sup>
Fluorescence <sup>b</sup>	Excitation, max = 335 nm Emission, max = 420 nm	N.D.
Cross reactivity to antisera to A-I, A-II, B, E, Lp(a)	none	none (3–5)
Carbohydrate (by weight)	6%	N.D.
Concentration in human serum (mg/dl)	13.1 ± 1.8 (n = 20)	14.2 ± 3.7 (5) (n = 59) 15.7 ± 0.9 (6) (n = 14) 14.1 ± 2.6 (10) (n = 5)

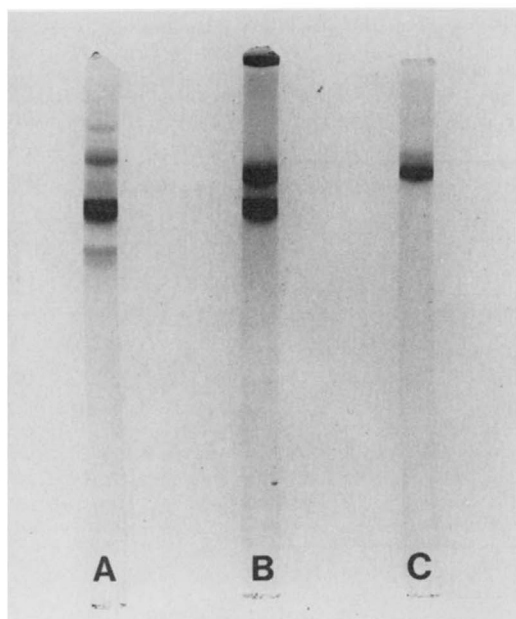
<sup>a</sup> Determined in 50 mM phosphate buffer, pH 7.8, 8 M urea, at a concentration of 0.743 mg/ml.

<sup>b</sup> Determined in 5 mM NH<sub>4</sub>HCO<sub>3</sub> at a concentration of 0.45 mg/ml.

<sup>c</sup> Determined after focusing in the presence of 8 M urea.

<sup>d</sup> Not determined.





**Fig. 4.** Analytic alkaline polyacrylamide electrophoresis in 7.5% gels in the presence of 8 M urea. A, Delipidated HDL; B, delipidated TRP following incubation with LPDS in 4 M NaCl; C, electroeluted apoA-IV. Twenty-five  $\mu$ g of protein solubilized in 5 mM Tris, pH 8.6, 8 M urea was applied to each gel.

SDS-free final product, without protracted diffusion dialysis. Nonetheless, protein adsorption to the dialysis sacs undoubtedly reduced the final yield.

The finding that apoA-IV isolated from LPDS is identical to apoA-IV isolated from lipoproteins implies that a chemical or structural alteration of this apoprotein does not occur attendant to its dissociation from the surface of chylomicrons and intestinal VLDL. The mechanism responsible for this dissociation has yet to be definitively established, although it is likely that the transfer of C apoproteins from HDL plays an important role.

Although most glycoproteins have relatively long half-lives in plasma, the half-life of human apoA-IV has recently been determined to be less than 24 hr (10), suggesting fairly rapid degradation at some undetermined site. It is likely that apoA-IV plays a regulatory role in lipoprotein metabolism, but further studies will be required to precisely define its metabolic function in circulation. ■

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